Kidney International, Vol. 48 (1995), pp. 903–910

Abstracts

South African Society of Nephrology

Endothelin-1 in renal diseases. *S. Naicker, R. Ramsaroop, P. Gathiram, A. Nadar, J. Duursma, V. Wilde, and I. Miyamori, Renal Unit, Addington Hospital and Department of Medicine, University of Natal Medical School, Durban, South Africa.* High concentrations of immunoreactive endothelin are present in the kidney. A variety of cell types (vascular endothelium, renal tubular cells, and mesangial cells) are able to produce endothelin. **Aims:** This study investigates the presence of endothelin-1 in plasma and renal tissue in renal disorders. **Materials and methods:** Frozen tissue from 15 renal biopsy specimens had immunohistochemistry performed on them using rabbit anti-endothelin-1 and a PAP procedure. Plasma levels of endothelin-1 were assayed by an ELISA technique (Takeda Chemicals, Japan). The diseases studied were primary mesangiocapillary glomerulonephritis (MCGN-3), mesangial proliferative glomerulonephritis (MGN-1), diffuse proliferative glomerulonephritis due to SLE (3), membranous nephritis (MN-2), IgA nephritis (2), acute tubular necrosis (ATN-1), and end-stage kidney disease (3). **Results:** There was intense staining of glomerular capillary endothelial cells and mesangial cells in MCGN, SLE, MGN and MN (1); intense staining of proximal tubular cells was seen in MCGN and SLE. Weaker staining was present in IgA nephritis, ATN and end-stage kidney disease and in endothelial cells of arterioles in all specimens. The results were similar in hypertensive and normotensive patients. Elevated plasma levels of endothelin-1 were present in patients with SLE and MCGN (3.0–4.5 pg/ml). Endothelin-1 levels in hemodialysis patients (without SLE) ranged from 1.4–2.0 pg/ml. **Conclusion:** Enhanced endothelin immunostaining was present in proliferative glomerulonephritides and SLE, with corresponding elevation of endothelin-1 in the plasma of these patients.

Hepatitis B-associated glomerulonephritis. A clinicopathological study in Namibian and South African children 1973–1993. *W.D. Bates, A.J. van Buuren, N. Muller, D.H. Geiger, and D.J. Rossouw, University of Stellenbosch and Tygerberg Hospital, South Africa.* Hepatitis B-associated glomerulonephritis is a common cause of nephrotic syndrome in children in endemic areas. The aim of this study was to determine whether this disease in children differs morphologically and clinically from the classical description of membranous nephropathy. The renal biopsies of 68 hepatitis-BsAg positive children (55 boys and 13 girls) were studied by light and electron microscopy as well as by immunological techniques. Twenty-seven of the children were Namibian and 41 from South Africa. At presentation all the children showed features of nephrotic syndrome. All biopsies showed some degree of mesangial deposits and proliferation, and 59 of the 67 (88%) with membranous features (subepithelial deposits) showed mesangial interposition. HB_cAg was demonstrated by monoclonal antibody in 87% of the biopsies tested. The membranous subgroup with severe mesangial interposition (23 cases) showed on biopsy (in comparison to the other 44 membranous examples) significantly more mesangial and subendothelial deposits, more advanced stages of membranous change, and more sclerosis. This subgroup was significantly older than the rest and hypertension was more frequent. All three of the membranous children who developed chronic renal failure, including 2 who died, belonged to this subgroup. The 41 Cape Province children showed an accumulated remission rate of 30% at 2 years and 63% at 4 years. Of the 68 patients, 4 are known to have developed chronic renal failure and of these 3 have died, one a Namibian child with a mesangiocapillary biopsy. Hepatitis B-associated glomerular disease in children is a unique form of glomerulonephritis which challenges and broadens the classical description, particularly of membranous nephropathy. Morphologically the glomeruli show mesangial deposits and mesangial interposition, which indicate a spectrum sharing features with mesangiocapillary glomerulonephritis. Clinically, the marked male predominance, and a high spontaneous remission rate of 60–90% over 4 years with a relatively low chronic renal failure rate and/or mortality (5–10%), distinguish it from the classical description of membranous glomerulonephritis.

A comparison of intravenous cyclophosphamide with oral cyclophosphamide for the treatment of lupus nephritis. Second interim report. *M.R. Moosa, A. Halland, C. Edelstein, and W. Bates, Department of Medicine, University of Stellenbosch, Renal Unit, Tygerberg Hospital, South Africa.* **Background and aims:** Lupus nephritis remains a potentially lethal complication of SLE in our experience. The treatment of lupus nephritis is a delicate balance between inadequate treatment with consequent renal loss and the complications of aggressive immunosuppressive treatment. We evaluated the clinical and pathological response to cyclophosphamide (CMP) therapy given either orally (POCY) or intravenously (IVCY) for a 2 year period, together with low dose steroids. **Methods:** Patients with newly diagnosed, biopsy proven, proliferative (WHO classes III + IV) and membranous (WHO class V) lupus nephritis were randomized to receive either POCY or IVCY. The dose of CMP was adjusted to maintain WCC 3–4000 ml. Clinical and biochemical parameters of response and adverse effects to treatment were monitored. The renal biopsy was repeated in all patients at 6 and 24 months. **Results:** Ten patients have thus far completed the protocol; 5 received POCY and 5 IVCY. The patients improved clinically and none suffered a flare-up of SLE. Biochemical and pathological data are shown below.

	Month		
	0 (IV/POCY)	6 (IV/POCY)	24 (IV/POCY)
Serum creatinine	132/105	89/82	133/87
DuProtein	1.0/2.3	0.1/0.9	0.5/0.7
C3	54/63	90/95	87/94
ESR	98/83	57/54	37/43
Activity index	6.2/6.8	2.2/3.2	2.6/3.0
Chronicity index	2.4/2.2	3.2/3.0	3.6/3.8

Results are presented as means. The improved results failed to reach statistical significance, but trends were demonstrated. One patient died during treatment and another required chronic dialysis after completion. Both were receiving IVCY. **Conclusion:** Combined steroid-CMP treatment is associated with marked improvement in lupus nephritis, with most of the improvement occurring in the first 6 months of treatment. No clear advantage of IVCY over POCY can yet be demonstrated. Serious complications, though rare, can develop with treatment.

Direct and indirect tests of pore size and charge selectivity in nephrotic syndrome. *G. Ramjee, H.M. Coovadia, and M. Adhikari, Department of Paediatrics and Child Health, Faculty of Medicine, University of Natal, Natal, South Africa.* **Introduction:** Disruptions of electrostatic charge barrier, pore size and shape of the glomerular basement are responsible for proteinuria in glomerular diseases. A similar negative charge is present on the membranes of red blood cells (RBC) and platelets. **Aim:** To use direct and indirect methods of membrane charge in the evaluation of proteinuria. **Methods:** Fixed anionic sites were detected using Polyethyleneimine (PEI) on the glomerular basement membrane (GBM) and Alcian blue on RBC membrane (ABRBC), respectively, in 33 children with nephrotic syndrome (NS). Size selectivity of the GBM was measured indirectly by fine analysis of urinary proteins using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS PAGE) in 22 of these children. **Main results:** Correlation between ABRBC and PEI was strongest ($r = 0.8$) in

4 children with minimal change NS (MCNS); moderate ($r = 0.31$) in 10 children with focal glomerulosclerosis (FGS); and absent in 14 children with HBs membranous nephropathy (MGN) and 5 with mesangioproliferative glomerulonephritis (MPGN). ABRBC and PEI were reduced in the group as a whole compared to their controls (40.87 ± 11.7 vs. 73.54 ± 13.14, $P < 0.05$; 15.75 ± 4.3 vs. 35.0 ± 1.2, $P < 0.005$, respectively). Excretion of glomerular proteins was restricted by size (≤ 80 kD) in MCNS, but unrestricted (≤ 80 kD plus > 80 kD) in FGS, MGN and MPGN. **Conclusion:** The main cause of proteinuria is likely to be depletion of negative charge on the GBM in MCNS, and distortion of capillary pore size in MGN and MPGN, with probable overlap of these mechanisms in each disease, especially in FGS. Basement membrane injury appears widespread in MCNS but confined to the kidney in MGN and MPGN.

Treatment of hyperlipidemia with simvastatin (SV) and diet, versus diet alone in idiopathic membranous nephropathy (IMN). *B.L. Rayner, M. Byrne, and R. van Zyl-Smit, Renal Unit, Groote Schuur Hospital, Cape Town, South Africa.* The aim of this study was to determine whether SV could safely lower cholesterol and retard progression of renal disease in patients with IMN. Patients with biopsy proven IMN, cholesterol >6.1 mmol/liter, 24 hour protein >3.5 g/day and creatinine <200 μmol/liter, were alternatively assigned to treatment with SV and diet, or diet alone. SV was begun at 10 mg/day and increased to a maximum dose of 40 mg until the cholesterol was <5.1 mmol/liter. Seventeen patients have been entered into the study. Mean follow-up was 18.8 months. Baseline data were similar between the groups and SV was well tolerated. The mean results for the first 18 months are shown below with the diet alone group shown first.

	Time, *months*				
	0	6	12	18	21
Albumin, *g/liter*	26.1	27.4	27.7	24.5	28
	25.6	24	30.1	43.2	41.3
uProtein/Creatinine ratio	0.68	0.55	0.81	0.74	0.55
	0.52	0.63	0.33	0.12	0.29
Cholesterol *mmol/liter*	10	10.0	9.6	9.7	10.5
	10.5	7.9	6.8	5.8	5.9

The rate of decline in Cr^{+++} EDTA clearance was similar between the 2 groups (−1.19 ml/min/month in the SV group and −1.35 in the diet alone group). In conclusion, this report suggests that SV can significantly lower cholesterol in nephrotic patients with IMN, and may reduce proteinuria and improve albumin levels.

Nephrotic syndrome in children at the H.F. Verwoerd hospital. *G. van Biljon, Department of Paediatrics, University of Pretoria, Pretoria, South Africa.* Sixty-seven children with nephrotic syndrome, presenting at the H.F. Verwoerd Hospital between June 1986 and June 1994, were investigated. Forty-four (66%) children were white, 14 (21%) were black, 3 (4%) were Asian and 6 (9%) belonged to the colored population group. Their ages ranged from 3 months to 12 years (mean 4.39 years); 74% of the children with minimal change nephrotic syndrome (MCNS) on histology were between 2–6 years old. Eleven children with clinical and biochemical data, in keeping with MCNS, did not undergo renal biopsy. They were presumed to have MCNS because of their previous or subsequent satisfactory response to corticosteroids. Added to the group with proven MCNS on histology, the total group constitutes only 56.7% of all patients, which is much less than the 76.4% reported by the ISKDC. Only two black children had MCNS, focal segmental glomerulosclerosis (FSG) being the most common histological feature in the black children. Eighty-one percent of children with MCNS responded well to corticosteroid treatment. Five children (18.5%) had early steroid resistance and five (18.5%) developed late steroid resistance. Corticosteroid treatment was of benefit to only one child with FSG. For the remaining 11 children with FSG, neither steroid nor cyclophosphamide treatment was of any benefit. Only two children had membranous nephropathy, both associated with HBs and HBe antigen carrier state. The incidence of this form of nephrotic syndrome is much lower compared to the report of Gilbert and Wiggelinkhuizen (16%) of Cape Town. Although congenital syphilis is still commonly seen in our neonatal wards, luetic nephrosis is rarely diagnosed. Only one of our patients had luetic nephrosis which responded poorly to penicillin treatment. Mortality in this group of 67 children was 3 (4.5%) and was due to end-stage renal failure.

The significance of arterial hypertension in patients with lupus nephritis (LN). *I.P. Naiker, V. Chrystal, I.G.H. Randeree, and Y.K. Seedat, Department of Medicine and Anatomical Pathology, University of Natal, Durban, South Africa.* This study aims to ascertain the prevalence of hypertension in patients with LN and to investigate a possible association between hypertension/renal vascular lesions and renal functional impairment. A correlation will also be sought between histological class of LN and hypertension. **Methods:** A retrospective analysis of 44 patients with a diagnosis of LN (WHO classification) was performed. Hypertension was graded as mild (diastolic 95–99 mm Hg), moderate (100–114), and severe (> 115). Impaired renal function (creatinine > 120 μmol/liter) was graded likewise. Histological class/presence of hypertensive renal vascular lesions (HRVL) was based on pathology reports. **Results:** Seventeen patients were hypertensive (group A); there was a prevalence of 38.6%. There were also 27 normotensives (group B). Incidence of renal impairment in group A versus B = 47% versus 18.5% ($P = 0.043$). Mean creatinine (μmol/liter) group A versus B = 283.4 versus 100.2 ($P = 0.023$). HRVL correlated with renal impairment; incidence 63.6% versus 18.1% ($P = 0.008$) and creatinine 392.7 versus 97.0 ($P = 0.012$). Severe (proliferative) lupus histology was more prevalent in group A, 70.5% versus 37.0% ($P = 0.030$). In group A there was no correlation between severity of hypertension and either renal function ($r = 0.089$) or histological class ($r = 0.078$). In conclusion, our results show a dissociation of hypertension and LN with a prevalence of only 38.6%. Hypertension and renal vascular lesions thereof correlated well with renal impairment. Although proliferative lupus lesions were more prevalent in hypertensives, there was no correlation between severity of hypertension and histological class.

Comparison of SDS PAGE of urinary proteins with the conventional method (selectivity index) for predicting steroid response in nephrotic syndrome. *G. Ramjee, H.M. Coovadia, and M. Adhikari, Department of Paediatrics and Child Health, Faculty of Medicine, University of Natal, Durban, South Africa.* **Introduction:** Steroids are the mainstay of treatment of nephrotic syndrome (NS) and have profoundly influenced management and outcome. Renal biopsy in these diseases depicts changes which correspond roughly to steroid responsiveness. However, biopsy is an invasive procedure and is not always reliable to judge therapeutic behavior to steroids. Accordingly, other predictive methods have been employed and the most widely used since 1966 has been the selectivity index (SI) measured by the ratio of IgG/transferrin in serum and urine. However, the validity of SI has been questioned in a number of studies and this has led to decrease in its use. We have recently shown that the qualitative detection of a range of proteins by sodium dodecyl sulphate polyarylamide gel electrophoresis (SDS PAGE) in the urine enables reliable prediction of steroid responsiveness. **Aim:** We compared the predictive powers of SI and SDS PAGE for steroid responsiveness in children with newly diagnosed minimal change nephrotic syndrome (MCNS) and focal glomerulosclerosis (FGS). **Methods:** There were 24 children with MCNS and 10 children with biopsy proven FGS. MCNS patients were those who responded to conventional doses and duration of steroid therapy and had a typical clinical course. All FGS patients were steroid resistant. SDS PAGE was carried out on a second morning sample of urine. SI was measured by nephelometry using simultaneously collected blood and urine samples. **Main results:** The positive predictive value for steroid responsiveness by SDS PAGE was 100% and that by SI 70%. The negative predictive value for steroid responsiveness was 100% by SDS PAGE and 40% by SI. **Conclusion:** SDS PAGE is a better predictor for steroid responsiveness than SI in MCNS and FGS.

Glomerular basement membrane charge in early onset pre-eclampsia. *T. Naicker, I.G.H. Randeree, J. Moodley, and Y.K. Seedat, Electron Microscope Unit, Natal Medical School, Durban, South Africa.* Early onset pre-eclampsia (EOPE) is a disorder characterized by severe proteinuria and hypertension before 34 weeks of gestation. Anionic sites on the glomerular basement membrane constitute an important barrier in ultrafiltration. Evidence suggests that the enzymatic removal or the neutralization of these sites results in proteinuria. A decrease in the number of anionic sites has been demonstrated in minimal change nephropathy and experimental aminonucleoside nephrosis. This prospective study was

undertaken to evaluate the fixed anionic sites on the GBM of African women with EOPE. **Method:** Staining of the anionic sites for electron microscopy, using a cationic dye (polyethyleneimine) was performed on kidney biopsies from 10 African patients with EOPE. Control specimens were obtained from patients requiring partial nephrectomy. **Results:** PEI labelled anionic sites revealed electron dense particles within the lamina rara externa (LRE), interna and lamina densa. The mean number of anionic sites per 1000 nm of LRE in the EOPE group was 19.21 ± 2.68 while that of the control group was 23.65 ± 0.74 ($P < 0.02$). The correlation coefficient of number of anionic sites versus severity of proteinuria was −0.79. **Conclusion:** This study, the first in African women with EOPE, demonstrates a significant reduction in glomerular basement membrane charge. The strong correlation between the severity of proteinuria and the loss of anionic sites in this study supports the hypothesis that loss of glomerular charge may be one of the mechanisms responsible for proteinuria in early onset pre-eclampsia.

Charge changes in early onset pre-eclampsia (EOPE). *I.G.H. Randeree, G. Ramjee, T. Naicker, J. Moodley, Y.K. Seedat, H.M. Coovadia, and M. Adhikari, Departments of Medicine, Paediatrics, Electron Microscope Unit, and the Medical Research Council Hypertensive Unit, Medical School, University of Natal, Durban, South Africa.* **Introduction:** Proteinuria in pre-eclampsia may result from altered shape, size or charge selectivity of the glomerular basement membrane (GBM). Charge selectivity is due to the presence of anionic sites on surface membranes. Both erythrocytes and the GBM possess similar anionic sites, allowing for indirect and direct assessment of charge changes. **Aim:** To assess membrane negative charge in EOPE using 2 cationic dyes, Alcian blue (AB) and polyethyleneimine (PEI). **Design:** Prospective descriptive study. **Subjects/methods:** *Indirect.* AB binding of erythrocytes: 11 patients with EOPE (24 to 34 weeks gestation) with 11 age, race and gestational age matched controls. *Direct.* PEI binding of the GBM: 10 patients with EOPE with 5 control specimens *viz.* post-traumatic partial nephrectomy. **Main outcome measures:** Alteration in AB binding of erythrocytes and PEI staining of GBM in EOPE patients compared to controls. **Results:** Indirect AB technique: AB binding was significantly decreased in the EOPE group 31.05 ng/10^6 cells compared to controls 65.12 ng/10^6 cells ($P < 0.0003$). Direct PEI technique: ultrastructural morphometric evaluation of the labelled anionic sites in the GBM showed a significant decrease in the lamina rara externa of the EOPE group compared to controls [19.21 vs. 23.65 ($P < 0.02$)]. **Conclusion:** The decreased binding of cationic dyes at the level of the erythrocytes and the GBM infers that a generalized disorder of membrane negative charge may exist in EOPE.

Correlation between histological changes and urinary patterns of proteinuria in early onset pre-eclampsia. *G. Ramjee, I.G.H. Randeree, J. Moodley, H.M. Coovadia, M. Adhikari, and Y.K. Seedat, Department of Paediatrics and Child Health, Faculty of Medicine, University of Natal, Durban, South Africa.* **Introduction:** Early onset pre-eclampsia (EOPE) is associated with a high incidence of underlying renal disease. Patients are generally not biopsied due to the invasive nature of the procedure. Accordingly, nephropathies may go undetected and appropriate therapy not instituted. This may lead to progression of underlying renal diseases. A non-invasive technique for predicting glomerulonephritis (GN) is by the use of sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS PAGE). Glomerular proteinuria is indicated by excretion of high molecular weight proteins and tubular proteinuria by excretion of low molecular weight proteins. **Aim:** To correlate the urinary patterns of proteinuria with histology in patients with EOPE and to evaluate the usefulness of this technique in predicting the presence of GN. **Method:** Ten patients with EOPE < 34 weeks gestation BP (≥ 140/90 mm Hg), proteinuria (> 4 g/24 hrs) and edema were studied by renal biopsy two weeks post-delivery. Urine analysis by SDS PAGE was carried out 48 hours prior to biopsy. **Main results:** Renal biopsy revealed underlying GN in 5 patients, and all 5 showed evidence of glomerular and tubular proteins. The other 5, without histological evidence of GN, had glomerular proteins only. **Conclusion:** SDS PAGE patterns of urinary proteins correlate well with histology and may be a reliable test for identifying underlying GN in EOPE.

Nuclear venography with technetium as a useful method for detection of subclavian vein obstruction. *A. Kobryn, J. Kowalczyk, and R.P. Clauss, Transplant Unit, Ga-Rankuwa Hospital, Pretoria, South Africa.* Subclavian vein obstruction is a well-known complication of prolonged catheterization of the subclavian vein for a temporary vascular access for hemodialysis. Formation of arterio-venous (A-V) fistula in the upper extremity in the presence of the subclavian vein obstruction results in a chronic swelling of the upper limb and progressive disability. To avoid these complications a routine screening of the renal failure patients with a history of the subclavian vein catheterization with Muharkar dual lumen catheter was carried out prior to A-V access formation. Our method selected for the investigation was the nuclear venography with GE Starport gamma camera and intravenously injected technetium. The following criteria for diagnosis of the subclavian vein stenosis or occlusion were defined: (1) the mean transit time through the subclavian vein above 10 seconds; (2) reflux of isotope to the unilateral jugular vein; (3) presence of a collateral circulation to the subclavian vein; (4) no flow of isotope through the subclavian vein. The study was performed in 78 renal failure patients. Seventy-two of them had undergone a unilateral or bilateral catheterization of the subclavian vein in the past. The mean dwell time of the catheter was 5 months. The subclavian vein stenosis or occlusion was found in 53 patients (71%), which resulted in selection of the contralateral upper extremity or the lower extremity for a permanent A-V access formation. **Conclusion:** The nuclear venography is a useful, non-invasive method for detection of the subclavian vein obstruction prior to A-V access surgery in renal failure patients with a history of the subclavian vein catheterization.

Coagulation profiles in chronic renal failure patients with subclavian vein thrombosis caused by prolonged catheterization with Mahurkar dual lumen catheters. *J. Kowalczyk, A. Kobryn, and G.A. Culligan, Transplant Unit and Haematology Department, Ga-Rankuwa Hospital, Pretoria, South Africa.* Hemodialysis via the subclavian vein catheter was developed as a temporary vascular assess for acute and chronic renal failure (CRF) patients prior to establishing permanent vascular access. Thrombosis of the subclavian vein after catheterization is a well-known late complication. Twenty-eight CRF patients from the northern Transvaal, who were being maintained on chronic hemodialysis at Ga-Rankuwa Hospital, were selected for the study. All the patients developed unilateral or bilateral subclavian vein stenosis following catheterization with Mahurkar dual lumen catheters. The average dwell time of a catheter was 5 months (5 weeks to 9 months). The diagnosis of thrombosis of the subclavian vein was established by both contrast venography and by technetium scanning in all patients. We performed coagulation profiles on these patients with the aim of establishing whether there was a hypercoagulable state that may contribute to the development of venous thrombosis. The following parameters were investigated: activated partial thromboplastin time (APTT), antithrombin III (AT III), protein C, protein S, fibronogen (Fib), and platelets. Samples of blood were taken prior to hemodialysis. The preliminary results are as follows:

APTT	34.3 (23.8–45.8)	N: 26–36 seconds
Fib	318.3 (210–500)	N: 150–400 mg/dl
AT III	74.9 (50–122)	N: 80–120%
Prot C	84.4 (42–109)	N: 70–140%
Prot S	79.1 (30–120)	N: 60–140%
Plat	186 (88–276)	N: 150–400 × 10^9/liter

Conclusions: The mean values of the studied parameters were all within the normal range with the exception of AT III. A slightly lower level of plasma AT III before hemodialysis is unlikely to be responsible for venous thrombosis alone. No episodes of deep vein thrombosis (DVT) were seen. The observed venous thromboses were restricted to the site of catheterization only. This suggests an involvement of local factors initiating a thrombotic process with, otherwise, normal coagulation state.

Acquired cystic kidney disease in hemodialysis patients. *L. Sofianou, V. Milkov, M. Rahme, and K. Missankova, Renal Unit, Department of Medicine, University of the Witwatersrand, Johannesburg, South Africa.* Twenty-eight patients on chronic hemodialysis for more than 3 years were assessed by sonography by 2 independent radiologists. Fourteen patients (5 females and 9 males) showed acquired cystic kidney disease (ACKD). All patients were asymptomatic-macroscopic hematuria, and severe flank pain was not diagnosed. None of these patients required any intervention. **Conclusion:**

Fifty percent of patients with end-stage renal disease on chronic hemodialysis for more than 3 years present with ACKD.

Investigation of calcium oxalate crystallization in urine using flow cytometry. *A. Rodgers, B. Hibberd, and T. Probyn, Chemistry Department, University of Cape Town, Rondebosch, South Africa.* **Aims:** It is widely recognized that an understanding of the mechanisms by which calcium oxalate crystallizes in urine is an essential prerequisite for understanding the morphogenisis of urinary calculi. Investigators have employed many different techniques for measuring *in vitro* crystallization processes and have identified nucleation, aggregation and growth as the key mechanisms. Unfortunately, in many cases it is not clear precisely which of these three mechanisms or, which combinations thereof, are being measured. In the present study we undertook to characterize calcium oxalate crystallization in urine by using flow cytometry. **Methods:** Twenty-four hour urine collections were obtained from 10 healthy male subjects (ages 20–27 years). Crystallization was induced in each urine by administration of an aqueous sodium oxalate load equivalent to the previously determined metastable limit. Crystallization in each specimen was monitored for 120 minutes by measuring particle number, size, and shape using flow cytometry. **Results:** Nucleation was identified as the sole crystallization mechanism in 7 urines. In the remaining 3 specimens, nucleation was accompanied by aggregation but not by growth. **Conclusions:** These results suggest that nucleation is the key mechanism by which urinary stones are initiated and that when crystallization occurs as a result of an increase in the relative supersaturation of calcium oxalate, growth does not appear to be important. The study has also demonstrated that meaningful results can be obtained using flow cytometry, thereby providing the stone researcher with a powerful tool for investigating urinary calcium oxalate crystallization processes.

Hematuria after megadose protocol of ascorbic acid. *B. Kroon, D. Auer, and A. Rodgers, Chemistry Department, University of Cape Town, Rondebosch, South Africa.* **Aims:** While several studies have suggested that vitamin C ingestion raises urinary oxalate, other studies have shown that raised levels might be due to *in vitro* conversion of ascorbate to oxalate. The present study was undertaken to examine the effect of vitamin C ingestion on oxalate excretion and calcium oxalate crystalluria. **Methods:** A 25 year old healthy male subject initially provided a 24 hour urine to serve as a control. Thereafter he commenced a protocol in which he ingested 9 g ascorbic acid per day for 8 days. Twenty-four hour urines were collected on days 1 and 5 of the protocol and on days 1 and 6 post-cessation of vitamin C ingestion. Calcium oxalate metastable limits, oxalate concentrations (in the presence and absence of EDTA), and ascorbic acid concentrations were determined in all specimens. Filtered crystals were examined by scanning electron microscopy (SEM). **Results:** The vitamin C protocol was originally planned to last 9 days. However, on the 8th day significant hematuria was detected and further vitamin C ingestion was stopped immediately. During the protocol, metastable limits decreased while the oxalate excretions, Tiselius risk indices, and calcium oxalate relative supersaturations increased. SEM revealed the presence of large crystal aggregates. **Conclusions:** The results indicate that vitamin C ingestion induced calcium oxalate crystalluria in this subject and that the passage of these crystals, as well as large aggregates thereof, caused irritation and epithelial injury manifesting as hematuria. Although this subject's response to vitamin C is probably rare, we believe that it should be brought to the attention of stone researchers as high dosage ingestion of ascorbic acid by such individuals could have very undesirable results.

Determination of functional renal reserve with radiopharmaceuticals. *G.P. Candy, J.D. Esser, D.R. Fine, and A.M. Meyers, Department of Nuclear Medicine, University of the Witwatersrand, Johannesburg, South Africa.* The renal functional reserve represents the capacity of the kidney to increase its level of operation under certain demands. The renal functional reserve can be determined by measuring the change in inulin or creatinine clearance following the ingestion of protein. The determination requires the continuous infusion of inulin and PAH for at least 6 hours. An increase of 20% in the glomerular filtration rate is regarded as normal renal functional reserve. **Aim:** An alternative to this is proposed in which the change in glomerular filtration rate [GFR (^{51}Cr-EDTA clearance)] and ^{99m}Tc-MAG clearance is determined before and after the consumption of a diet high in protein for up to 7 days. **Methods:** The patients (10) selected for the study were potential renal donors undergoing routine evaluation of renal function. Baseline GFR was determined by following the clearance of ^{51}Cr-EDTA from the blood by taking multiple blood samples up to 210 minutes after injection. ^{99m}Tc-MAG was injected simultaneously and MAG clearance (approximately 2/3 of ERPF) was determined simultaneously from the activity in the same blood samples. The patients were requested to consume a diet high in protein (meat, fish) for up to 7 days. The GFR and ^{99m}Tc-MAG clearance was determined again. Each determination was carried out in the morning following a light (no meat), caffeine-free breakfast with the patient hydrated. Results are shown in the table.

Table 1. Change in GFR and ^{99m}Tc-MAG clearance following protein loading

	GFR		^{99m}Tc-MAG clearance	
Patient	*N*	*ml/min*[a]	*N*	*ml/min*[a]
Pre-protein loaded	10	90.7 ± 23.7 (47.8–129.4)	6	220.5 ± 23.7 (185.2–264.7)
Post-protein loaded	10	113.1 ± 27.1 (74.8–158.3)	5	539.3 ± 183.8 (324.7–784.1)
% increase[a]		24.7 ± 12.8 (12.9–55.0)		128 ± 87 (44.6–269)

[a] Data are mean ± SD (range)

Conclusions: (1) GFR increased by 12–55% following the consumption of a diet high in protein, similar to literature values. (2) Increases in ^{99m}Tc-MAG clearance paralleled the increase in GFR, and this finding is in keeping with a reported increase in PAH clearance following a protein load. (3) The proposed method of determining the renal functional reserve appears considerably simpler than the conventional inulin infusion.

Geriatric CAPD in Johannesburg—Joys and jeopardy. *K.I. Furman and A.M. Meyers, Department of Medicine, Division of Nephrology, University of the Witwatersrand and the Johannesburg Hospital, Johannesburg, South Africa.* From July 1979 to June 1994, a total of 89 patients over the age of 60 years were started on CAPD. Ages ranged from 60 to 82 years with a mean of 64.7 years. Duration of treatment varied from 3 to 128 months, with a mean of 27.2 months. Thirty-three (38%) of these patients were on CAPD for less than 1 year and a further 17 (19%) for less than 2 years. Twenty-two (25%) were on CAPD for more than 3 years and 13 (15%) for over 5 years. The mortality rate was understandably high for this age group. Forty-nine (55%) died while on CAPD, and 25 of these died within the first year on CAPD, mostly due to co-morbid pathology that was either unsuspected or considered to be stable and controlled at the start of treatment. Five patients died from peritonitis. Eight patients were transplanted, six of whom died within 2 years post-transplantation. Thirteen patients were transferred to hemodialysis because of repeated attacks of peritonitis or inadequate ultrafiltration. All have since died, as did 7 patients in whom all treatment was withdrawn because of inability to cope with the regimen. Six patients were transferred to other centers and were lost to follow-up. In the remainder, CAPD was adequate and effective in achieving a good measure of rehabilitation. Spouse and family support played a major role in correcting errors of technique and instilling confidence to these elderly patients who frequently became forgetful and confused.

Nutritional assessment and adequacy of dialysis in CAPD patients. *C. Willett, M. Hollander, E. de Reuck, G. Fortuin, and B. Rayner, Renal Unit and Department of Dietetics, Groote Schuur Hospital, Cape Town, South Africa.* Adequate nutrition and adequacy of dialysis are important determinants of morbidity and mortality in CAPD patients. Our aim was to prospectively study this in our CAPD population. All new CAPD patients, who had not previously undergone long-term hemodialysis or renal transplantation, were studied. At entry all patients had standard peritoneal equilibration tests and 24 hour dialysate and urine collection to calculate their weekly creatinine clearance, KT/V urea, and protein nitrogen appearance (PNA). Additionally, trained dieticians (CW and MH) did anthropometric measurements to calculate fat free mass, % body fat and BMI, and took detailed dietary histories. These measurements were all repeated at 6, 12 and 24 months. Only data are included of

patients completing more than 6 months of follow-up. To date 7 patients have been followed for 6 months and a further 6 for 12 months. There are 2 males and 10 females with a mean age of 52 years. Anthropometric data tended to show a gain in body weight due to an increase in % body fat. Fat free mass remained stable. Patients tended to decrease their energy intake due to a reduction in carbohydrate intake. Most patients had adequate weekly creatinine clearance, KT/V urea and PNA, but these declined with time due to a reduction in residual renal function. There was good correlation between KT/V urea, weekly creatinine clearance, and PNA. The creatinine ratio at 4 hours correlated well with KT/V urea and creatinine clearance. In summary, most CAPD patients following standard dialysis prescriptions had no evidence of significant malnutrition, or evidence of inadequate dialysis.

Assessing the adequacy of CAPD. *G.H. Latiff, S. Naicker, Y.K. Seedat, and G. Nel, Renal Unit, Addington Hospital, and Department of Medicine, University of Natal, Medical School, Durban, South Africa.* **Aims:** (1) To assess, using KT/V urea, whether our patients are being adequately dialyzed. (2) To calculate, using urea kinetics, the protein catabolic rate (PCR)—a measure of the dietary protein intake (DPI). (3) To determine the peritoneal membrane permeability of these patients using a 4 hour peritoneal equilibration test (PET). **Methods:** Twenty-five stable CAPD patients were randomly selected. A KT/V urea of 1.7 per week was defined as minimum acceptable therapy. A PCR of 1 g/kg/day was regarded as adequate. Depending on the results of the PET, patients were divided into 4 categories according to high, high average, low average and low transporters—according to the rate of transport of glucose and creatinine (expressed as ratios) across the peritoneum. All calculations were done using a computer-based kinetic modeling program for peritoneal dialysis (PD ADEQUEST). **Results:** Seventeen males and 8 females comprised the study sample. Six (24%) patients had KT/V values of less than 1.7. Fifteen (60%) patients had a PCR of less than 1 g/kg/day. Based on PET results, 14 (56%) patients were "average" transporters, while 5 (20%) and 6 (24%) patients had high and low permeability membranes, respectively. **Conclusions:** (1) ± 25% of our CAPD patients are being underdialyzed. Quantitative assessments of the adequacy of dialysis need to be routinely implemented, allowing for a prospective and objective approach to dialysis. (2) Protein mulnutrition is a major problem in our unit, with 60% of patients having a subnormal DPI. Urgent attention needs to be paid to dietary counseling and adjustments of dialysis regimens. (3) Most patients were "average" transporters and would be suitable for any form of peritoneal dialysis. High and low transporters require special dialysis prescriptions.

Bilharzia and renal failure in Natal. *N.G. Christopher, J.C. Oviedo-Pascottini, S. Naicker, and K.N. Rughubar, Department of Urology, Department of Medicine, and Department of Pathology, University of Natal, Durban, South Africa.* **Introduction:** The current impression in South Africa is that the bilharzia seen here differs from that in Central Africa and Egypt. Progressive disease leading to renal failure has been reported as rare by Powell et al (1968). Naude (1984) concluded that, "in Natal, bilharzia of the ureter has been found to be surprisingly harmless". The clinical impression is that these conclusions are no longer valid. **Aim:** (1) A preliminary study to assess the number of patients treated with renal failure and bilharzia. (2) To assess whether renal failure is due to mechanical obstruction or due to glomerulonephritis as seen with *Schistosoma mansoni.* **Methods and materials:** A prospective study between July and December 1992. All patients with bilharzia and renal failure were assessed. Patients with reflux were excluded. Patients' age, sex, full blood count, urea and electrolytes, creatinine, creatinine clearance, C3 + C4 complement, and immunoglobin were recorded. Renal and ureteric specimens were sent for histology. Rectal biopsies were taken to exclude *S. mansoni.* **Results:** In 1992, 15 patients with bilharzia were treated for renal failure. Ages ranged from 5 to 36 years (mean 21 years). Male to female ratio was 9:6. Africans to Indians ratio was 14:1. Seven patients were studied, 11 renal biopsies/nephrectomy specimens were analyzed from July–December 1992. Serum complement and immunoglobins were normal in all. Rectal biopsies excluded *S. mansoni.* Renal histology included tubular atrophy, periglomerular fibrosis, glomerular fibrosis, chronic pyelonephritis, and interstitial nephritis. The ureters showed fibrous replacement of smooth muscle. **Discussion:** Kruger (1990) has shown that limited hybridization S. hematobium/S. mattheei does not influence the virulence of the parental species as previously postulated in South Africa. In this preliminary study, bilharzial ureteric strictures caused renal failure by mechanical obstruction. This needs to be confirmed in a larger series.

Prolonged survival of children with autosomal recessive polycystic kidney disease (ARPKD) for more than 18 years. *K.E.C. Meyers and P.D. Thomson, Division of Paediatric Nephrology, Department of Paediatrics, University of the Witwatersrand and Johannesburg Hospital, Johannesburg, South Africa.* **Aim:** To assess the long-term survivors with ARPKD. **Method:** A retrospective chart review of children at our institution surviving for 18 years or more with ARPKD. **Results:** There were 9 patients for analysis, 5 of whom were male. Their ages ranged from 19.85 to 24.45 years. Three patients died at 19.85, 20.58, and 24.25 years, respectively. Age range at diagnosis was 0.1 to 11.85 years (2.86 ± 4.17). Hepatic manifestations were central hepatomegaly and hepatic fibrosis (9), splenomegaly with portal hypertension (7), porto-systemic shunt procedure (5), gastro-esophageal varices (2), and splenectomy (2). One patient had ascending cholangitis. Renal complications included, large kidneys (9), hypertension (8), urinary infections (3), and native nephrectomies (1). Time from diagnosis to onset of ESRF was 4.25 to 16.82 years (10.99 ± 4.62). Eight of nine patients were transplanted (TP). TP_1 LRD = 3; TP_1 CD = 5; TP_2 All CD = 4. The pre-emptive transplant status was: PD 0/8; HD 2/8; "off the floor" 6/8. Actuarial first graft survival was: 1 year 100% and 4 years 69%. Immunosuppression included: Pred/Aza (2/8); Pred/Aza/CSA (5/8); plus TLI (1/8). Growth was generally better post-transplant.

	Pre-TP		TP	Post-TP	
YEARS	−3	−1	0	+1	+3
Mean Δ VSDS	−1.09	−1.21	−2.42	1.21	3.20

The three patients who died were complicated by hepatic encephalopathy, variously raised ammonia levels, and normal synthetic function/enzymes. **Conclusions:** The long-term prognosis for ARPKD is not as poor as originally reported. Late liver decompensation is a problem. Because of the latter, hepato-systemic shunting must be considered with circumspection, particularly if liver-renal transplantation is to be contemplated. Further studies of ammonia handling by children with ARPKD are needed.

A scoring system for evaluating patients for selection to a dialysis/transplant program. *M.L. Griffiths and C.F. Kewley, Renal Unit, Frere Hospital, East London, South Africa.* **Aim:** To assess the feasibility of a point system to evaluate prospective dialysis and transplant candidates. **Methods:** Using a questionnaire, points were allocated to patients with chronic renal disease in 3 categories, according to: (1) Communication, insight (ability to speak English, standard of education, availability of telephone). (2) Access to the dialysis center (distance, transport). (3) Socio-economic factors (employment, housing, support system). To test the reliability of the scoring system, 20 previously accepted patients were analyzed retrospectively. **Results:** Complete data were obtained in 99 patients. Sixty-five patients were aged 59 years or less. Of these, 34 (52%) were in stable employment, 32 (49%) had completed primary school, and 40 (62%) were able to communicate in English, and 39 (60%) lived within 50 kilometers of the center. Associated diseases which could adversely affect selection were present in 25 (38%). The most common was diabetes (16 patients). With our limited resources ideal candidates were considered to be 49 years or less, with stable employment, had passed Std 5, were able to speak English and had no other serious disease. Only 17 patients of the total sample (17%) met all these criteria, and 7 of these resided within 50 kilometers and the other 10 resided more than 50 kilometers from the dialysis facility which would virtually exclude them. These two groups of patients scored an average of 20 and 14.3, respectively. In the retrospective analysis, a score of 17 or more predicted satisfactory compliance. **Conclusion:** The scoring system predicts successful compliance on a dialysis and transplant program. More than half (58%) of the ideal candidates lived further than 50 kilometers from the hospital, and these areas require separate dialysis facilities.

The correction of the anemia of chronic renal failure with recombinant human erythropoietin (rHuEPO)—Stabilization and maintenance study.

M.R. Moosa, C.R. Swanepoel, C.L. Edelstein, and J. Jacobs, Renal Unit, Tygerberg Hospital and the Department of Internal Medicine, University of Stellenbosch, Cape Town, South Africa. **Introduction and aims:** Anemia, which is present in 97% of patients on maintenance hemodialysis, can be seriously debilitating. The major cause of the anemia is the failure of erythropoietin production by the diseased kidney. Recombinant human erythropoietin (rHuEPO) can produce a dose-related rise in the hemoglobin level with an associated improvement in symptomatology related to tissue hypoxia. The aim of this study was to determine the efficacy and safety of a locally produced rHuEPO product (Repotin). **Method:** Patients on hemodialysis who had hemoglobin levels of less than 8 g% and satisfied all the inclusion criteria were entered into the study. Repotin was administered at an initial dose of 25 IU/kg intravenously three times per week. The dose was increased until a target Hb of 8 g/dl was reached. The Repotin was then administered subcutaneously to maintain Hb levels of 9–10 g/dl. The patients were carefully monitored for any side-effects. **Results:** Twenty-seven patients were successfully enrolled and followed-up in the study. The mean Hb rose from 6.55 g/dl to 8.07 g/dl over a period of 12 weeks. The mean dose of Repotin used was 63.4 IU/kg. In the maintenance phase 55.5 IU/kg of Repotin was required to maintain the target Hb between 9–10 g/dl. A total of 46 adverse events was recorded in 21 patients. Only 15% were thought to be causally related to the treatment. The most serious of these adverse events was the clotting of 3 fistulae. **Conclusion:** Repotin is an effective and safe rHuEPO product.

A low-dose strategy for erythropoietin (EPO) in a large dialysis unit. *A.A. Khan, R.F. Jeffrey, P. Prabhu, A.M. Davison, and E.J. Will, St. James's University Hospital, Leeds, England, United Kingdom.* **Aims:** The optimum strategy for EPO use in a large dialysis unit is undefined. We have investigated the hypothesis that fixed low-dose subcutaneous (s.c.) EPO would obviate the need for transfusion, and effect a favorable hematological response, with minimal supervision. **Methods:** Twenty-five transfusion-dependent adult dialysis patients (18 hemodialysis, 7 CAPD) received a fixed dose of 1,000 units EPO thrice weekly. Monthly hematological profiles and transfusion requirements before and after EPO treatment were recorded. Patients were reviewed monthly and side-effects noted. Duration of treatment ranged from 12–105 weeks (mean 59). **Results:** Three patients did not respond to EPO because of chronic GI blood loss, chronic sepsis, polyserositis, and Al toxicity. The remaining 22 responded with an increase in Hb [6.86 ± 0.9 g/dl (SD) to 9.06 ± 1.18 g/dl (SD) after 3 months). Nine of the 22 patients achieved a Hb of 10 g/dl or more after a mean of 18 weeks (range 8–52). Ninety-four u packed cells were transfused to the 22 responders during the 3 month period to EPO treatment and there were no transfusions during the first 3 months of treatment. Mean BP did not change during treatment. Two patients developed hypertension, one of whom clotted her fistula. **Conclusions:** In the group where no EPO resistance factor was present, fixed low dose s.c. EPO conveyed hematological benefit and, in view of a favorable side-effect profile, would allow reduced medical review. This implies that a higher proportion of patients in a large dialysis unit could be treated safely and with benefit, than if an individual high-dose strategy were adopted.

Intravenous iron in hemodialysis patients. *R. van Zyl-Smit and J.A. Halkett, Renal Unit Groote Schuur Hospital and Department of Medicine University of Cape Town, South Africa.* Iron supplementation is often ineffective in correcting iron deficiency in hemodialysis patients. We report the successful use of total dose iron infusion (TDI) of an iron polymaltose complex in 59 patients with minimal adverse effects and a gratifying therapeutic response. TDI was given to patients with a hemoglobin of <10 g/dl and a transferrin saturation of 20% or less. Despite receiving 200 mg of ferrous sulphate after each dialysis, 36% of our screened hemodialysis patients were iron deficient by these criteria with a mean transferrin saturation of 11% and mean ferritin levels of 68 ng/ml. Of these, 37% responded with a rise in hemoglobin of 2 g/dl or greater [the average rise being 3.16 g/dl, reaching a level of 9.8 g/dl (range 6.9–13.7 SD 1.88)]. A lower mean baseline hemoglobin prior to TDI was predictive of a good response. There was no difference in baseline mean ferritin values between responders and non-responders (66 vs. 71 ng/ml). Similarly, no difference in mean baseline transferrin saturation was noted (10.9% vs. 12.1%). This study demonstrated the high frequency of iron deficiency, despite oral supplementation, the use of transferrin saturation as a marker of iron deficiency, and the safety and efficacy of the particular intravenous iron preparation used.

Leukocyte analysis of tubulointerstitial nephritis in primary mesangiocapillary glomerulonephritis (MCGN). *I.P. Naiker, R. Ramsaroop, S. Somers, I.G.H. Randeree, S. Naicker, and Y.K. Seedat, Departments of Medicine and Anatomical Pathology, University of Natal and Regional Laboratory Services, Durban, South Africa.* The pathogenesis of tubulointerstitial inflammation in relation to primary glomerular diseases is uncertain. It may progress, however, perhaps due to cellular immune mechanisms and may be the key to progression to chronic renal failure. This study aims to characterize the mononuclear leukocytic interstitial infiltrate in primary MCGN and correlates the degree and nature of infiltrate with renal function methods. Cellular identification using monoclonal antibodies to leukocyte cell-surface antigens was conducted on tissue from 17 patients with primary MCGN (Group I) and 10 controls with non-proliferative GN (Group II); renal function was estimated at the time of biopsy. **Results:** Group I showed significant increase in total leukocytes (LCA), T lymphocytes (T), and B lymphocytes (B) compared to Group II. Monocytes (KP_1) were not significantly increased. Cells/mm^2 (Group I vs. Group II): LCA 1306 ± 1254 versus 138 ± 91 ($P = 0.0004$); T 638 ± 547 versus 42 ± 60 ($P = 0.005$); B 161 ± 165 versus 57 ± 50 ($P = 0.02$); and KP_1 125 ± 153 versus 54 ± 59 ($P = 0.17$). In Group I, T cells were dominant (that is, 48.8% of total, B 12.3%, and monocytes 9.5%). A moderate correlation existed between LCA and impaired renal function: serum creatinine ($r = 0.43$) and creatinine clearance ($r = -0.41$). This correlation was strongest for T cells ($r = 0.63$) followed by KP_1 ($r = 0.46$) and B ($r = 0.41$). In conclusion, we have demonstrated a significant mononuclear leukocytic interstitial infiltrate in primary MCGN and a correlation between all cells of this infiltrate with impaired renal function. This correlation was strongest for the most frequently observed cell, the T lymphocyte.

Preliminary experience with FK 506 in renal allograft rejection. *M.J.D. Cassidy, M.D. Pascoe, E.R. Irving, and D. Kahn, Department of Medicine and Surgery, University of Cape Town and Groote Schuur Hospital, Cape Town, South Africa.* FK 506 is a potent immunosuppressive agent which has similar actions and side-effects to cyclosporine A. Though structurally dissimilar, they are both derived from a fungal source. Since February 1994 we have used FK 506 (Fujisawa GmbH) as rescue therapy on compassionate grounds in 7 patients with steroid resistant acute renal allograft rejection. The group consisted of 3 females and 4 males with a mean age of 30 years (range 22–49); baseline immunosuppression consisted of cyclosporine A, prednisone, and azathioprine. In 6 patients this was the first graft. In 5 cases FK 506 was used as rescue therapy in the first 3 weeks after transplantation and in the other 2 cases at 9 and 53 months, respectively. In all cases acute rejection was not controlled by pulse solumedrol, 500 mg daily on three successive days. OKT3 was not used due to fluid overload at the time of rejection in all cases. Acute severe rejection was confirmed in all cases histologically. Rejection was reversed in 6 of the seven patients, with a current mean serum creatinine 266 μmol/liter (range 141–419); 3 patients have a current serum creatinine of < 200 μmol/liter. Dialysis was stopped in the seventh patient for a short period after commencing FK 506; however, adequate graft function was not maintained and a graft nephrectomy was performed 1 month after commencing treatment. No clinically significant side-effects were encountered. In conclusion, our preliminary experience with the use of FK 506 rescue therapy has been extremely encouraging in patients suffering from steroid resistant acute allograft rejection.

Combination therapy with ketoconazole and cyclosporine in renal transplantation. *M. Pascoe, J. Halkett, M. Cassidy, and D. Kahn, Renal Unit, Groote Schuur Hospital, and Departments of Medicine and Surgery, University of Cape Town, Cape Town, South Africa.* The concomitant use of CsA with ketoconazole (K) alters the metabolism of CsA such that lower doses of CsA may be used to maintain therapeutic blood concentrations. In the 3 year period up to December 1993, 32 renal allograft recipients received combined cyclosporine and ketoconazole therapy (C/K), and a group of 34 patients served as controls. The 2 groups were similar in age, sex, and primary renal disease. The C/K group received combination therapy for 12 months, while the control group received CsA for 6 months and then prednisone/azathioprine only. Patients were followed from the time of grafting to death, or until December 1993. The mean follow-up for the C/K group was 22.5 ± 10.2 months and for the controls was 32.9 ± 18.5 months. C/K therapy was prematurely stopped in 4 patients, 1 because of poor control of hypertension, 2 because of difficulty with control of CsA

blood concentrations, and 1 at the patient's request. Death due to sepsis occurred in 3 patients in the first 3 months post-transplantation, and 1 patient lost the graft 9 months post-transplant while on maintenance with prednisone/azathioprine only. In the control group 5 patients died of a variety of causes. Graft function was lost in eight patients a mean of 52 weeks post-transplant (range 1 day to 3 years). The renal function was not different between the 2 groups 12 months post-renal transplant. The combination of CsA with ketoconazole can be used as a cost effective method for reducing immunosuppressive costs and making the benefits of CsA available to patients at substantial cost savings.

Organ procurement in the Johannesburg area. *L. Botha, M. Veller, R. Britz, and J.R. Botha, Clinic Holdings Limited and J.H.B. Transplant Unit, South Africa.* The number of the patients in end-stage renal failure has reached dramatic proportions in South Africa. An increasing number of cadaver donors is currently required to make renal transplantation available to these patients. This retrospective study was undertaken to investigate the (1) number of renal donors available, (2) their source, and (3) the subsequent distribution of the procured kidneys in the Johannesburg area. In the 18 month period, January 1, 1993–June 30, 1994, 69 renal cadaver donors were harvested in the Johannesburg area. All harvesting and transplantation was performed by the Johannesburg hospital transplantation surgical team. The median age of the donors was 21 years with a range of 2 to 63 years, and the male/female ratio was 3:1. The causes of death were: trauma (52), natural death (14), overdose (2), and carbon monoxide poisoning. 1. The blood groups were, Gr-A: 24 patients; Gr-B: 9 patients; Gr-O: 3 patients; Gr-AB: 3 patients; and unknown in 2. Of the 69 donors, 34 (49%) were from the private sector and 35 (51%) were from Johannesburg hospital. In the six months of 1994, there were 16 (64%) donors from the private sector and 9 (36%) from the Johannesburg hospital. During the period studied, 118 kidneys were transplanted in the Johannesburg area, 10 (9%) in the private sector, and 101 (91%) at the Johannesburg hospital. **Conclusion:** (1) There is an increasing number of donors available in the private sector and a changing pattern of donor availability in Johannesburg. (2) Close cooperation between the private and public sector will be necessary to effectively utilize all potential donors in the Johannesburg area.

Attitude toward organ donations and transplantations in the Eastern Cape. *V. Ramiah, D. Walker, T. Maweni, and D. Kahn, Livingstone Hospital, South Africa.* **Aim:** To assess the attitude, awareness, and acceptability of organ donation and transplantation among Xhosa speaking black patients in the Eastern Cape. **Method:** One hundred thirty-five Black patients were interviewed by a Xhosa speaking Sister in GOPD at Livingstone Hospital. **Results:** Forty-eight percent of those interviewed had heart about brain death. Only 18%, however, would accept that a family member was dead if brain death was diagnosed; 95.5% had heard of organ donation and 62% of patients interviewed would give consent to donation of family members' solid organs. Only 35% of the total would give consent for donation of eyes. A reported 78.5% of patients would have a transplant if they required one; 81% would allow and advice a relative to have a transplant if required. Only 15.5% would accept a transplant from an animal. **Conclusions:** More than one-half of the study group were aware and would consider organ donation. Transplantation is acceptable to the majority of the group studied. Although the term brain death is familiar with the majority, the actual concept is not properly understood. Education of the community in order to dispel myths and fears cannot be emphasized enough. There is a definite, strong resistance to xenografts.

Immunolocalization of atrial natriuretic peptide and tissue kallikrein in the transplant kidney during acute rejection. *D. Moodley, C. Snyman, S. Naicker, R. Ramsaroop, and K.D. Bhoola, Department of Experimental and Clinical Pharmacology, Natal Medical School, Durban, South Africa.* Atrial natriuretic peptide (ANP), a hormone with natriuretic and diuretic properties, plays a pivotal role in renal sodium and water homeostasis. ANP has been immunolocalized in the intercalated cells of the connecting tubules of the kidney and tissue kallikrein (TK) in the connecting tubule cells, which is considered to increase vascular permeability through the release of kinins. As yet there is no evidence as to which of these proinflammatory peptides are responsible for the vascular and cellular damage observed during renal transplant rejection. Our study therefore examines the spatial and cellular distribution of renal ANP and TK, in normal and rejection states. **Materials and methods:** Renal biopsies from patients with acute rejection, taken as a diagnostic procedure, were fixed in 10% normal saline and embedded in paraffin wax. Normal kidney was obtained from cadaver specimens. ANP and TK were immunolocalized using antibodies specific to human ANP (1:100) and human TK (1:100). The antibodies were preabsorbed with human IgG and human albumin (1 mg/ml) to reduce non-specific labelling. For the immunolabelling controls, primary antisera were replaced with buffer or non-immune serum. **Results:** When compared to the normal renal tissue, ANP immunoreactivity in the acutely rejecting transplant kidney was considerably depleted or absent in the intercalated cells of the connecting tubule. Importantly, good labelling results were obtained for TK in both the normal and rejecting kidney. No labelling was observed in the method control sections. **Conclusion:** ANP secretion in the acutely rejecting transplant kidney is reduced, or even absent, when compared to the normal tissue. However, no obvious reduction in the immunolabelling of TK was found. Our results seem to indicate that ANP may be involved in the primary rejection process. However, the question whether excessive formation of kinins contributes to the rejection process remains unresolved.

An outbreak of nocardiosis in a renal transplant unit. *G.H. Latiff, S. Naicker, Y.K. Seedat, A.A. Haffejee, and Y.M. Coovadia, Renal Unit, Addington Hospital, and Departments of Medicine, Surgery and Microbiology, University of Natal, Medical School, Durban, South Africa.* **Aims:** To describe an outbreak of nocardia infection in our transplant unit. **Methods:** The medical records of 9 patients who acquired nocardiosis between August 1991 and June 1993 were retrospectively reviewed. All were recipients of first renal allografts. **Results:** The study sample was comprised of 6 males and 3 females (mean age = 40 years). Seven received cadaver kidneys and the remaining 2 were living transplants. The mean period between the date of the transplant and the onset of symptoms was 34 weeks. Symptoms were non-specific. All patients had pulmonary disease. Eye disease with subsequent blindness and cutaneous involvement (breast abscess) were seen in 2 patients, respectively. Radiological features included cavitation, consolidation, and pleural effusions. Examination of sputa for typical branching filaments led to a diagnosis within 48 hours of admission. Diagnosis was subsequently confirmed by culture in all patients. All patients were successfully treated with Co-Trimoxazole. Attempts to isolate a hospital source of the infection were unsuccessful. **Conclusions:** (1) A high index of clinical suspicion is essential for early diagnosis. (2) Sputum examination was a reliable and quick method of diagnosis. (3) It is unlikely that these patients acquired their infection in the hospital.

Pregnancy after kidney transplantation. *J. Kowalczyk, A. Kobryn, D. Mahapa, and M. Marivate, Transplant Unit, Ga-Rankuwa Hospital, Pretoria, South Africa.* From January 1984 to December 1993, 98 kidney transplants were performed at the Transplant Unit, Ga-Rankuwa Hospital. Thirty-four recipients were female patients. Fifteen recipients of childbearing age underwent the study. All 15 patients started to menstruate regularly after kidney transplantation, and while on hemodialysis only 2 experienced irregular menstrual bleeding. Five out of 15 patients planned pregnancy after transplantation, and 2 of them successfully delivered healthy baby boys. Two recipients had miscarriages and 1 experienced intrauterine fetal death at 7 months of gestation. The first patient became pregnant 7 years after a cadaveric kidney transplantation. Prior to pregnancy, her graft function was stable with a serum creatinine of 98 μmol/liter. The immunosuppressive regimen consisted of azathioprine (Aza, 2 mg/kg body wt) and low dose prednisolone (Pred, 0.2 mg/kg body wt). The second patient became pregnant 6 years after a cadaveric kidney transplantation, having a stable graft function and a serum creatinine of 120 μmol/liter. She has been maintained on tripple immunosuppressive therapy with cyclosporine A (8.5 mg/kg body wt), Aza (2.0 mg/kg body wt) and Pred (0.1 mg/kg body wt). Both babies were delivered by Caesarean section with an Apgar score of 9. There were no congenital abnormalities found. At pediatric follow-up the babies are well at 30 and 6 months post-delivery, respectively. **Conclusion:** Kidney transplant offers a significant improvement of quality of life to most chronic renal failure patients. A risk of immunosuppression seems to have no major influence on the outcome of pregnancy or child development.

Risk factors in acute renal failure—A prospective 5-year study (449 cases). *C.D. Potgieter, Division of Nephrology, University of Pretoria, Pretoria, South Africa.* **Aim:** A prospective 5 year study evaluating several risk

factors relating to the morbidity and mortality of acute renal failure. **Methods:** Each case of acute renal failure referred to the renal service was evaluated. The following factors were recorded: causes of acute renal failure, initial urea, creatinine and electrolytes, blood pressure, fractional sodium excretion, renal sizes, liver functions, time to recovery or death, presence of oliguria or anuria, episodes of hypotension, cardiovascular factors, respiratory factors, gastrointestinal factors, surgical factors, treatment modalities, and outcomes. **Results:** Mortality was ± 63%. The following risk factors were of major importance in influencing the mortality: pneumonia, ventilation, hypotension, sepsis, diffuse intravascular coagulation, etc. **Conclusions:** Several factors influencing the survival of patients with acute renal failure are important in evaluating patients and in planning appropriate therapy. Risk factor analysis needs to be done in each case to provide optimum therapy.

Allopurinol for calcium oxalate stones: The right drug for the wrong reason? *P.K. Grover, V.R. Marshall, and R. Ryall, Department of Surgery, Flinders Medical Centre, Bedford Park, South Australia, Australia.* A critical appraisal of the evidence commonly cited to support a link between high urate excretion and calcium oxalate (CaOx) urinary calculi will be presented. Analysis of relevant literature has revealed that this is based on the clinical impression that stone formers tend to excrete more urate than do healthy subjects and that administration of allopurinol reduces, albeit slightly, recurrences of urinary calculi in patients with "hyperuricosuria." Two theories have been invoked to provide a scientific explanation for urate's apparently promotory effect. The first proposes that urinary urate crystals promote CaOx precipitation by the phenomenon of epitaxy; the second hypothesizes that particles of urate reduce the inhibitory activity of urinary glycosaminoglycans which normally prevent the crystallization of CaOx. However, to the present day, neither has been experimentally verified. This has led to skepticism about the existence of a relationship between a high urinary urate excretion and CaOx stone formation in general, and the therapeutic use of allopurinol in particular. More recent research from our group has revealed that at normal physiological pH values, dissolved urate directly promotes CaOx precipitation by the classic "salting-out" effect by enhancing nucleation, growth, and aggregation of CaOx crystals. Evidence linking urate excretion to CaOx urolithiasis will be presented and discussed. It will be shown that the beneficial effect of allopurinol can be attributed to its lowering the urinary output of urate and thereby reducing the probability that CaOx will be salted out of urine, rather than to epitaxy or inactivation of urinary glycosaminoglycans. Thus allopurinol may, in fact, be the right drug for preventing stone recurrences—but for the wrong reason.

The Banff classification of renal transplant pathology: Clinicopathological correlation. *R. Ramsaroop, S. Naicker, G.H. Latiff, and A.A. Haffejee, Regional Laboratory Services, and Renal Unit, Addington Hospital, Durban, South Africa.* Renal transplant biopsies are required for accurate diagnosis and treatment of graft dysfunction, especially following the utilization of cyclosporine (CsA) immunosuppressive regimens. The reporting of such biopsies has previously been subjective and the Banff Working Classification of Kidney Transplant Pathology was recently formulated as an international standard. This study is an audit of renal transplant biopsies over a six year period, from July 1988 to June 1994, reclassifying the histology according to the Banff Classification. Clinicopathological correlation relating histological severity to outcome is included. A total of 176 biopsy and 20 nephrectomy specimens from 116 patients was reviewed. The histological categories included rejection (135) and other pathology (29). Rejection was further categorized into acute rejection (AR) (103)—Grade I (60), II (32) and III (11); chronic rejection (CR) (14)—Grade I (12) and Grade III (2). Twenty of the AR fell into a borderline category, where the histologic changes were patchy and early, and 2 showed hyperacute rejection. Other pathology included: CsA toxicity (4), acute pyelonephritis (3), interstitial nephritis (6), and acute tubular necrosis (6). Graft loss was recorded in 30 patients, the most frequent reason being infarction following acute vascular rejection (12). Other reasons included: CR (6), progressive AR (4), recurrent glomerulonephritis (2), and severe pyelonephritis (2). Most of the patients (58) with stable renal function showed borderline to Grade I AR, with only 2 with evidence of CR. Patients with gradual deterioration in renal function totalled 10, with 6 showing a progression from acute to chronic rejection on biopsy and 4 showing mild acute rejection with concomitant severe interstitial nephritis. **Conclusions:** (1) We have found that the Banff Working Classification has promoted standardization of histologic criteria in allograft biopsies. (2) It may serve as a guide to therapy. (3) There was good correlation between histologic severity and clinical outcome.

Effect of urinary prothrombin fragment 1 on calcium oxalate crystallization in undiluted human urine. *R.L. Ryall, P.K. Grover, A.M.F. Stapleton, Y. Tang, R. Moritz, and R.J. Simpson, Department of Surgery, Flinder Medical Centre SA, and Ludwig Institute for Cancer Research, Victoria, Australia.* Urinary prothrombin fragment 1 (F1) is the predominant protein incorporated into calcium oxalate (CaOx) crystals precipitated from human urine. The aim of this study was to examine the effect of pure F1 and CaOx crystallization in undiluted human urine. CaOx crystals were generated in urine obtained from healthy men, by the addition of oxalate, and demineralized with EDTA. The resulting extract was then desalted and purified by reversed phase liquid chromatography on a Brownlee RP-300 microbore column. Additional 24 hour urine samples were collected from healthy men, pooled, centrifuged (10,000 × g) and 0.22 filtered (0.22 μm). A portion was retained as control (c) and the remainder was ultrafiltered (10 kD; UF) and divided into aliquots to which was added F1 at final concentrations of 0, 1.25, 2.5 and 10 mg/liter. CaOx crystallization was then induced in each sample by the addition of oxalate, followed by incubation with shaking for 2 hours at 37°C. The size and volume of the particles deposited were determined using a Coulter Counter and the crystals were examined by scanning electron microscopy (SEM). CaOx precipitation was also determined in parallel experiments by 14C-oxalate deposition. The amount of oxalate required to induce crystallization was identical in all samples, but the volume of particulate material deposited increased in a dose-dependent manner from 8,154 $\mu m^3/\mu l$ in UF urine to 16,814 $\mu m^3/\mu l$ in the UF urine containing 10 mg/liter of F1. The highest volume, 23,450 $\mu m^3/\mu l$, was recorded in the C urine. However, the mean particle size decreased in reverse order from 18.3 μm in the UF to 7.266 μm in the UF + 10 mg/liter urine, and 8.462 μm in the C urine. This decrease in size was confirmed by SEM, which showed that it was the result of a reduction in crystal aggregation rather than in the size of individual crystals. Analysis of 14C-oxalate data revealed a dose-dependent decrease in CaOx deposition with an increase in F1 concentration, indicating that the increase in particle volume recorded by the Coulter Counter resulted from inclusion of F1 into the crystalline architecture, rather than increased deposition of CaOx. It was concluded that F1 is the most potent macromolecular inhibitor of CaOx crystal growth and aggregation in undiluted urine yet detected, and may therefore be an important macromolecular determinant of stone formation.

Minimal change nephrotic syndrome (MCNS)—A 12½ year review at Baragwanath Hospital. *U.K. Kala and D.W.C. Jacobs, Department of Paediatrics, Baragwanath Hospital, University of the Witwatersrand, Johannesburg, South Africa.* **Aim:** To analyze the incidence and response to therapy in black African children at Baragwanath Hospital from July 1981 to December 1993. **Methods:** A retrospective analysis of all patients with biopsy proven MCNS with respect to age of presentation, sex, microscopic hematuria, hypertension, response to steroids and cyclophosphamide, the need for a second biopsy and incidence of pulmonary tuberculosis and renal failure. **Results:** MCNS form 23.9% (82) of all nephrotics. Mean age of presentation was 6.1 years (range 1.25–11.9 years). Male:female ratio was 2.2:1. Incidence of microscopic hematuria was 84.1% (69). Initial hypertension occurred in 13.4% (11); 2.4% (2) had steroid related hypertension and 4.9% (4) had hypertension requiring therapy. Of 96 biopsies, 81 were initial, 15 (18.3%) repeat, and 2 post-mortem biopsies. Of the repeat biopsies 8 had focal glomerulosclerosis, 6 remained MCNS, and one was familial, 2.4% (2) had acute renal failure at presentation and 2.4% (2) developed chronic renal failure. Spontaneous remission in (6.1%), 5 steroid responsiveness initially in (68%) 56, partial response in (6.1%) 5 and non-responsiveness in (13.4%) 11. Frequent relapses occurred in (25%) 14 of initial responders and of 12 treated with cyclophosphamide 7 responded. TB occurred in 11% (9). The mortality was 4.9% (4). Thirty-eight patients have been lost to follow-up. **Conclusion:** MCNS accounts for 25% of the total nephrotics under 12 years. There is a high incidence of microscopic hematuria at presentation. Initial response to steroids conforms to that reported internationally.